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Avoidance of Copper and Nickel by Rainbow Trout as Monitored by a Computer-Based Data Acquisition System

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16. ABSTRACT
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Avoidance of Copper and Nickel by Rainbow Trout as Monitored by a Computer-Based Data Acquisition System

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Abstract

The avoidance response of rainbow trout *Salmo gairdneri* to copper and nickel solutions under several exposure regimes was evaluated with a computer-based data acquisition system interfaced with a linear, plexiglass chamber with countercurrent water flow. The best estimates of the avoidance thresholds for these metals were 6.4 μg/liter total copper and 23.9 μg/liter total nickel (95% confidence limits were 2.6–15.5 and 10.2–54.3 μg/liter, respectively). For each metal, avoidance threshold concentrations were not different whether fish were exposed in shallow or steep concentration gradients. Also, no significant differences ($P > 0.05$) were found when fish received multiple, increasing exposures or a single exposure in steep-gradient conditions. Rainbow trout initially avoided low copper concentrations, but were attracted to higher ones (330–390 μg/liter) during shallow-gradient tests. Under similar test conditions, fish were attracted to low nickel concentrations (about 6 μg/liter), but avoided higher levels (>19 μg/liter). The 24-hour average concentration of these two metals presently considered adequate for the protection of freshwater aquatic life fell within the 95% confidence limits for threshold avoidance concentrations reported here. This suggests that environmental impacts predicted on the basis of toxicity tests alone do not reflect potentially important behavioral changes caused by subchronic concentrations of copper and nickel. Avoidance tests, therefore, may prove to be a valuable tool for screening toxic chemicals, providing additional information and a broader perspective for evaluating the impact of aquatic contaminants on fishery resources. The results indicate that the steep gradient, in which a single fish is exposed to several consecutively increasing doses, provides the simplest means of exposure, yielding easily quantified information amenable to statistical analysis. The computer-based system also provides a rapid, easy-to-operate, accurate, and relatively inexpensive means of collecting avoidance data.

At present, the minimum data base required for setting water-quality criteria for a given substance is founded primarily on acute and chronic toxicity testing with several species representing several trophic levels. No standard system or operating procedure has been adopted for conducting behavioral tests. The primary reasons have been difficulties in quantifying behavioral responses, variability in the measured end points, uncertainty as to the relative significance of behavioral responses in comparison to other lethal and sublethal indicators of stress, and the uncertainty of extrapolating laboratory results to field situations. Our study addressed these problems as they relate to the behavioral responses of rainbow trout *Salmo gairdneri* to copper and nickel.

The ability of fishes to avoid extreme temperature, heavy metals, pesticides, chlorine, and a variety of other substances has been documented (for example, Sprague and Drury 1969; Hansen et al. 1972; Cherry, et al. 1977; Cherry et al. 1979; Giattina et al. 1981). Attention has been focused on the avoidance response because of its importance to fish and other organisms that are capable of increasing their chances of survival by moving away from harmful conditions. In theory, any substance present in sufficient quantity or under certain environmental conditions may render a habitat unsuitable, so that fish avoid it and a fishery resource is lost. For this reason, possible behavioral effects should be considered when water-quality criteria are established, and the preference or avoidance responses of fish would be of obvious importance given the effects these behaviors have on fish distributions.

There may be no particular relationship, however, between behavioral responses of an organism to a pollutant and the toxic effects of

that pollutant. For example, Sprague and Drury (1969) reported that near-lethal concentrations of detergent and phenol were not avoided by rainbow trout, but this species did avoid zinc sulfate at concentrations lower than one-tenth of the incipient lethal level (Sprague 1968). Avoidance responses of fishes to potential aquatic contaminants, therefore, can provide pertinent information on the environmental impact of a substance which is not readily apparent from classical toxicity tests.

Several basic laboratory methods have been used to determine avoidance behavior. Experimental designs range from highly automated computer-based systems (for example, Kleerekoper et al. 1972; Cripe et al. 1975; Westlake and Lubinski 1976; Cripe 1979; Lang$_1$ et al. 1979; Lang$_2$ et al. 1979) to simple experimenter observation (for example, Sprague 1964, 1968; Hansen 1969; Sprague and Drury 1969; Hansen et al. 1972, 1974; Cherry$_2$ et al. 1977; Cherry et al. 1978, 1979). Also, a variety of chamber designs have been used (circular, Y-shaped, linear). This diversity of systems often makes comparison of results difficult, emphasizing the need for a standard testing scheme which would give meaningful results. To evaluate the feasibility of standardizing the avoidance test for use as a hazard assessment tool, we have developed a computer-based data acquisition system for monitoring fish reactions to potential aquatic contaminants. The purpose of this study was to examine the avoidance response of rainbow trout to copper and nickel under several different exposure conditions and, based on the evidence collected, to suggest a feasible means of conducting future avoidance testing. These metals are on the United States Environmental Protection Agency's (USEPA) list of 65 priority pollutants for which water quality criteria documents have been written (USEPA 1980a).

Methods

Water Quality and Test Organism

All avoidance tests were conducted at the Western Fish Toxicology Station (WFTS), a facility of the USEPA's Corvallis Environmental Research Laboratory. Acclimation and testing were conducted in WFTS well water which was aerated and temperature-controlled at 12 ± 1 C. Means (SE) for hardness, alkalinity, and dissolved oxygen were 28.4 (1.4) and 25.2 (1.0) mg/liter $CaCO_3$, and 10.3 (0.04) mg/liter, respectively (N = 57). The mean pH was 7.3 with a range of 7.0–7.5 (N = 54). The test water is further described by Samuelson (1976).

Rainbow trout were chosen as the test organisms because considerable supporting data are available from chronic and acute toxicity tests on this species. Green eggs were obtained from a local fish hatchery and incubated at 8 C at WFTS until hatching. Eggs were disinfected with malachite green at least 3 months prior to testing and young fish were treated with Furanace® to control fungal and bacterial infections. Avoidance tests were begun about 3 months after swim-up. Fish (mean wet weight = 10 g; mean fork length = 7 cm) were fed Oregon Moist Pellet to repletion 2–3 times per week during the testing but were not fed while in the avoidance chamber. All fish were temperature-acclimated at least 1 week before exposure, and were kept under natural lighting and photoperiod conditions.

Toxicants

Nickel chloride ($NiCl_2 \cdot 6H_2O$) and cupric chloride ($CuCl_2 \cdot 2H_2O$) were used in the avoidance tests; all concentrations are reported as total metal. Although little research has been conducted on the behavioral effects of nickel, copper has been extensively studied and was examined here to facilitate comparisons between the present experimental design and those used by previous researchers.

Stock solutions were prepared by dissolving the appropriate salt in 80 liters of WFTS well water, with 0.1 ml/liter concentrated nitric acid (HNO_3). Samples for metal analysis were taken in acid-washed containers and acidified by the addition of one volume percent of concentrated nitric acid. Metal analyses were carried out with the furnace technique for atomic absorption spectrophotometry outlined by the USEPA (1979). Due to the low organic content of the test water, however, an ashing time of 10 seconds at a temperature of 1,000 C was used instead of the 30 seconds–900 C regime proposed. Analytical precision was ±1 μg/liter for both copper and nickel.

Water Delivery and Avoidance Chamber

Avoidance responses of individual fish were evaluated for three exposure conditions: (1) a steep chemical gradient in which the metal concentration was increased in magnitude over

time; (2) a steep gradient in which a fish was exposed to a single concentration; and (3) a shallow chemical gradient in which the metal concentrations were increased in magnitude over time. With each regime, a sharp interface was maintained between control and toxicant water so that a choice was available to the fish.

The avoidance chamber was a linear, acrylic trough (20.5 × 20.5 × 137 cm) with water entering each end and draining from the center. Stainless steel screening was inserted into each end creating an oval-shaped swimming area so fish could not linger in corners. Well water was delivered to the chamber from six overhead mixing boxes, which were fed dilution water from a single, constant-head-pressure, 80-liter aquarium (Fig. 1). Toxicant and control (0.10 mg/liter HNO_3) stock solutions were pumped from two 80-liter aquaria and injected directly into the mixing boxes feeding the ends of the trough. Velocity of flow in the chamber varied from approximately 6 to 17 cm/minute depending on the type of test conducted.

The steep chemical gradient was formed by allowing dilution water plus the toxicant solution to enter the ends of the chamber only. This incoming toxicant concentration remained essentially constant until water exited at the center drain (Figs. 1 and 2). Flow patterns were identical in the control end of the chamber. The magnitude of the toxicant gradient was increased with time, so each fish tested was exposed to several increasing concentrations, or a single fish could be exposed to only one concentration and discarded.

The shallow chemical gradient was formed in the chamber by introducing a toxicant into one end and dilution water at points along the side of the trough. This dilution water entered the trough below a perforated false bottom 5 cm above the floor of the tank, and was forced up through 2-mm-diameter holes located in a row at the point of entry of the dilution water (Fig. 1). This current of clean water was broken by a fine gravel overlay which increased the turbulence and enhanced mixing of the dilution with the treated water. The toxicant solution was diluted twice in this manner before exiting out the center drain, forming three areas differing in concentration (Fig. 2). As in the steep-gradient tests, a fish was exposed to a gradual increase in the toxicant concentrations with time. The pattern of water flow was identical in the control end of the chamber. All flow patterns were confirmed with methylene-blue dye studies; toxicant dilutions are illustrated in Fig. 2.

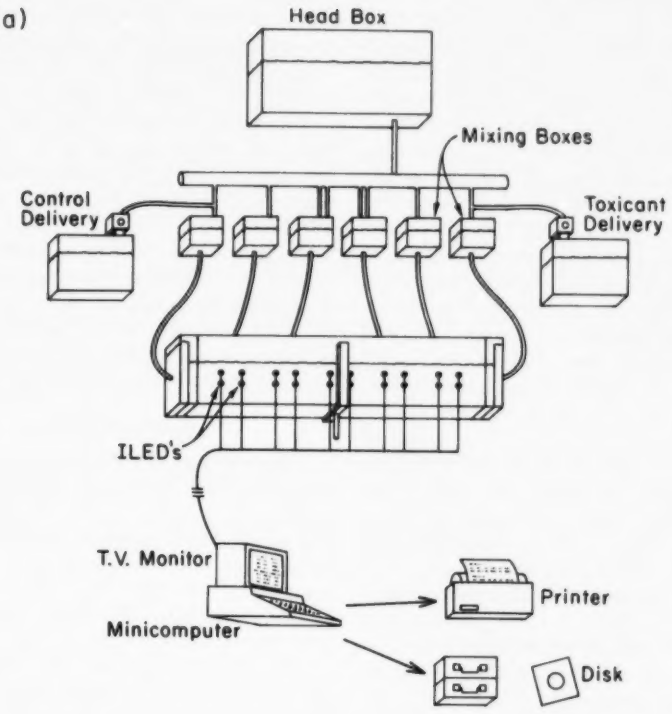
The avoidance chamber was completely surrounded by a plywood enclosure that had two sliding doors for access. The inside was painted flat black. Water samples were collected from tygon tubes connected to the trough, and running to the outside of the enclosure so that samples could be taken without disturbing the fish. Lighting was provided by a single 40-watt incandescent bulb located directly over the center of the chamber, and two fluorescent tubes located over each side of the chamber. These lights were suspended approximately 0.5 m above the trough and behind a sheet of opaque plexiglass.

Computer Interface

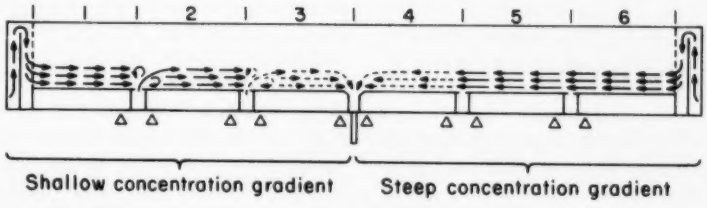
The computer-based data acquisition system automatically collected data from the avoidance chamber by monitoring 20 infrared light-emitting diode (ILED)-phototransistor pairs, thereby tracking the movement of a fish during testing (ILEDs—RCA SG 1009A; phototransistors—Motorolla MRD 3055). Two ILEDs and two phototransistors were held in identical acrylic frames with attached convex lenses (diameter = 27 mm; focal length = 35 mm) (Fig. 1). The lens on the ILED side of the chamber collimated light emitted from an ILED; the one opposite focused this light onto the phototransistor surface after it passed across the avoidance chamber. The two ILEDs and phototransistors, when suspended from opposite sides of the chamber, formed a vertical plane or "gate" of infrared light. Ten such gates were placed along the trough and corresponded to a particular zone of the trough (Fig. 1). A fish blocking either of the infrared light paths of a gate caused the transmission of one +5-volt signal to a custom-built interface card in the computer. Each gate was scanned every 100 milliseconds and the results of the scan were converted into the position of the fish within the chamber and stored in a data array.

The minicomputer system consisted of an Apple II Plus computer with 48K bytes of random-access memory and 12K bytes of read-only memory. A real-time clock card maintained continuous time and date information. Hard copies of all data were obtained from an Anadex Model DP-800 AP line printer. The data-acquisition system was alternatively powered by

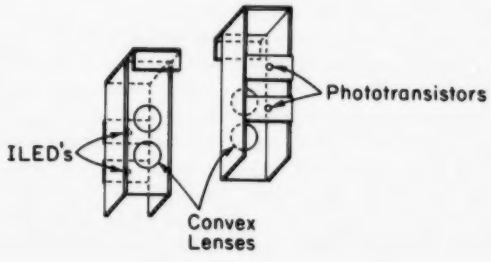
(a)


Head Box
Mixing Boxes
Control Delivery
Toxicant Delivery
ILED's
T.V. Monitor
Minicomputer
Printer
Disk

(b)


1
2
3
4
5
6
Shallow concentration gradient
Steep concentration gradient

(c)


Phototransistors
ILED's
Convex Lenses

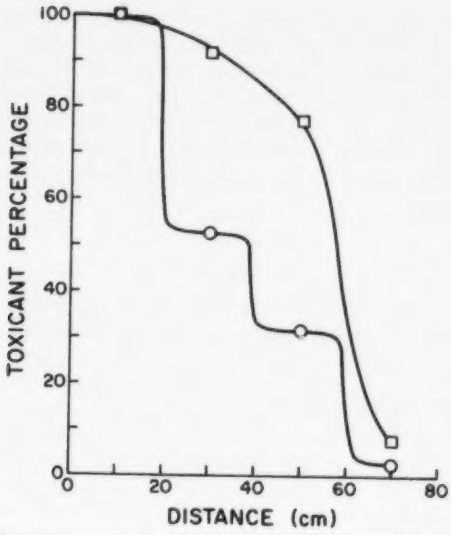


FIGURE 2.—*Measured concentrations of toxicant on the treated end of the avoidance tank expressed as percentages of the initial concentration entering the end of the chamber. The center drain was located at 60 cm (shallow gradient ○; steep gradient □).*

a 12-volt battery which could sustain operations 20–40 minutes in the event of a power failure. Total cost of this system and the avoidance trough was approximately $5,500.

The data collected were the amount of time (seconds) that the fish spent in each of the six zones and the number of times each infrared gate was blocked. The latter values were used to estimate the activity of the fish during a test. Data were displayed on the computer video monitor (Video 100) along with a moving asterisk that corresponded to the location of the fish. The cumulative time and activity of a fish were transferred every 10 minutes from computer memory to a data file on a magnetic diskette for permanent storage.

Testing Procedure and Statistical Analysis

A single fish was removed from its holding pen (a temperature-controlled tank located outside the laboratory under natural lighting conditions) and placed in the avoidance chamber. When the fish had begun exploratory behavior (some time during a 30–60 minute adjustment period) and was spending about equal time on each side of the chamber, toxicant delivery started. The last 10-minute interval before toxicant delivery served as the control time for the particular fish. This control period was followed by a 10-minute toxicant introduction period, during which either a shallow or steep gradient was formed; this was followed by 10 minutes of data collection at the first exposure level. Thus, each fish was exposed to each concentration for a maximum of 20 minutes. This procedure was followed for each of the 2–4 exposure levels applied to each fish. Introduction of the toxicant from either end of the chamber was alternated after every five tests. Between tests the avoidance chamber was drained and dilution water was allowed to rinse the system for a minimum of 15 minutes. Water samples were taken periodically from the exposure end of the chamber prior to toxicant introduction to insure that no metals remained from the previous test. Tests generally were conducted twice daily, one in the morning and one in the afternoon.

In tests with the steep gradient, the assumption of independence of error terms was in question, because the response of a fish at higher test concentrations may have been influenced by its exposure to the previous, lower concentrations. To evaluate this possibility, two series of steep-gradient tests were conducted under identical conditions. In one series, a minimum of eight fish were individually exposed to four increasing concentrations; in the other series, a minimum of four fish were individually exposed to only one concentration and discarded.

Upon completion of both steep-gradient tests, the amount of time a fish spent in the treated area and its activity (number of blocks per gate) were determined by summing these values in each of the three zones of the treated end of the chamber. The proportional change in the

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FIGURE 1.—*Schematic representation of the avoidance system: (a) diagram of water delivery system, avoidance chamber with infrared light-emitting diodes (ILEDs), and computer monitoring system (phototransistors not shown); (b) diagram of avoidance chamber showing water-flow patterns under shallow- and steep-gradient conditions (open triangles indicate position of ILED gates); (c) diagram of ILED "gate" showing vertical positions of ILEDs, phototransistors, and convex lenses. Ten such gates were placed along the chamber to track the movement of a fish.*

amount of time (PC_{time}) a fish spent in a treated area compared with control conditions was then calculated from

$$PC_{time} = \frac{TIME_{treatment} - TIME_{control}}{TIME_{control}}$$

where $TIME_{treatment}$ is the number of seconds a fish spent in a toxicant concentration, and $TIME_{control}$ is the number of seconds spent in the area prior to toxicant introduction. A similar calculation was made to determine the proportional change in activity ($PC_{activity}$). The PC values were averaged for each concentration and plotted against the mean log toxicant concentration associated with the change; regression analyses then were performed. The average PC_{time} values calculated were either positive (indicating attraction to the toxicant), negative (avoidance), or zero (no change), and the magnitude of the value reflected the strength of the response. Differences between the regression lines generated for each of the steep-gradient tests were evaluated by analysis of variance.

The avoidance threshold was defined as the concentration that caused a 50% reduction in the amount of time a fish spent in an area relative to respective control times. This value was estimated from the regression line, and 95% confidence limits were calculated.

To insure that time in the avoidance chamber did not significantly influence fish behavior, a separate series of control tests were conducted by the procedures outlined above for the multiple-exposure steep-gradient tests. No toxicant was added but time and activity were recorded for the fish at the same time intervals as in the metal tests.

In tests conducted with the shallow gradient, the amount of time a fish spent in any area of the toxicant gradient may have been influenced by the toxicant concentration in an adjacent area. The concentration–response relationships, therefore, could not be analyzed statistically because the underlying assumption of independence of error terms for each variate may have been violated. For this reason, a qualitative graphical approach was used to determine the avoidance responses of rainbow trout under these conditions. A frequency distribution comparing the mean total residency time fish spent in each concentration was plotted with the mean toxicant concentration corresponding to that zone. Fish would, in general, spend approximately 100 seconds in each of the six zones during a 10-minute control time. The avoidance threshold, therefore, was defined as the first toxicant concentration associated with a decline in the residence time below 50 seconds because this would be about a 50% reduction and, hence, comparable to the results from the steep-gradient tests.

Results

Steep-Gradient Tests

The residence time and activity of rainbow trout in treated areas was inversely related to toxicant concentrations tested under steep-gradient conditions with multiple exposures (Fig. 3). The multiple-exposure threshold values determined for copper and nickel were 6.4 and 23.9 μg/liter, respectively (95% confidence limits [CL] were 2.6–15.5 and 10.2–54.3 μg/liter). Rainbow trout avoided test concentrations of copper and nickel above 6 μg/liter, spending over 30% less time in them (Table 1). The attraction to 6 μg/liter nickel (noted later) in shallow-gradient tests was not evident in steep-gradient tests; several fish showed some indication of attraction, but the general response was slight avoidance ($PC_{time} = -0.06$) at this concentration.

The dose-response lines generated from the steep-gradient tests with fish receiving a single toxicant exposure were not significantly different ($P > 0.05$) from those lines generated from multiple exposures (Table 1; Fig. 3). The single-exposure threshold values for copper and nickel were 7.3 and 24.5 μg/liter, respectively. Increased variation around the regression line was noted in single-exposure ($r^2(Cu) = 0.43$; $r^2(Ni) = 0.80$) as compared with multiple-exposure steep-gradient tests ($r^2(Cu) = 0.84$; $r^2(Ni) = 0.93$). For nickel, this reduction in the explained variation was caused by a somewhat ambiguous response to concentrations in the range of 15–28 μg/liter.

The influence of previous exposures on avoidance to higher concentrations appears negligible. Thus, violation of the assumption of independence of error did not appear to considerably influence subsequent results, indicating that multiple exposure of the same fish to increasing concentrations of copper and nickel is a valid procedure, particularly when short time intervals of exposure (20 minutes or less) are used. However, given longer exposure in-

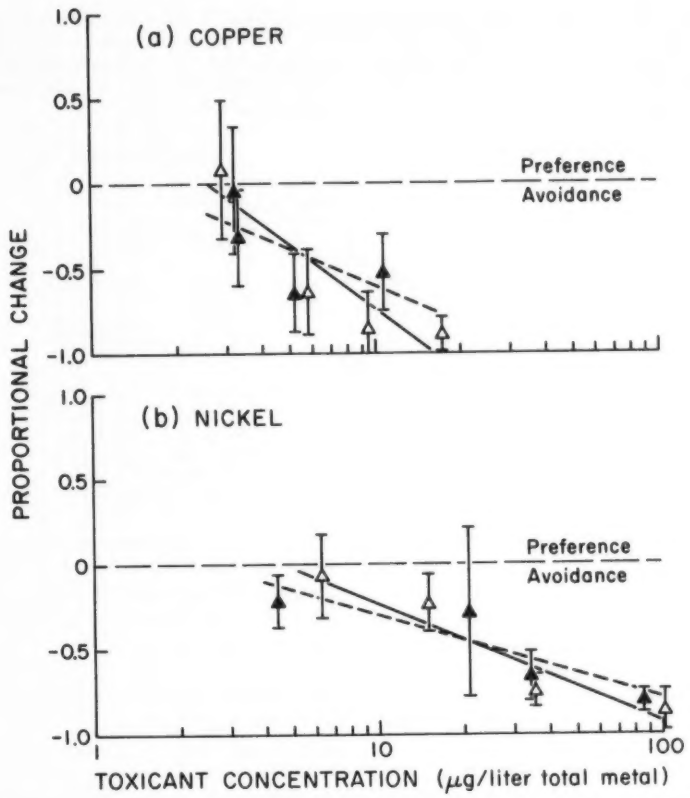

FIGURE 3.—*Mean proportional change in residence time (PC_{time}) and toxicant concentration of rainbow trout tested in single (▲- - -▲) and multiple (△——△) exposure, steep-gradient conditions for copper (a) and nickel (b). Regression equations for single and multiple exposures to copper are* $PC_{time} = -0.72\ log_{10}[Cu] + 0.12$ ($r^2 = 0.43$; *threshold value = 7.3 μg/liter) and* $PC_{time} = -1.26\ log_{10}[Cu] + 0.51$ ($r^2 = 0.84$; *threshold value = 6.4 μg/liter), respectively. Regression equations for single and multiple exposures to nickel are* $PC_{time} = -0.48\ log_{10}[Ni] + 0.17$ ($r^2 = 0.80$; *threshold value = 24.5 μg/liter) and* $PC_{time} = -0.70\ log_{10}[Ni] + 0.47$ ($r^2 = 0.93$; *threshold value = 23.9 μg/liter). Vertical bars are ±2 standard errors.*

tervals, possible complicating factors such as acclimation may influence behavior at higher test concentrations and significantly alter avoidance thresholds.

Activity changes for fish tested under steep-gradient conditions were closely correlated with changes in residence time as toxicant concentrations increased (Table 1). At the lowest copper concentrations, however, activity for some fish was increased in both single- and multiple-exposure tests, although the PC_{time} was relatively unchanged. This indicated possible stimulatory effects on locomotion at these low concentrations (2.9–3.2 μg/liter), most likely a reflection of either increased swimming speeds, higher frequencies of entry into the toxicant, or both. This effect was not observed in nickel tests or shallow-gradient copper tests.

Control Tests

Time in the avoidance chamber was not a significant factor affecting fish behavior. Regression analysis of the PC_{time} and $PC_{activity}$ did not reveal significant slopes with time for either variable, although there appeared to be a trend for activity to gradually decline (Table 2). The PC_{time} and $PC_{activity}$ during the control tests and the multiple-exposure, steep-gradient

TABLE 1.—*Means (SE) of the proportional changes in residence time (PC_{time}) and activity ($PC_{activity}$) of rainbow trout associated with geometric means (SE) of toxicant concentrations. The steep-gradient condition, in which a single concentration was present in half of the avoidance chamber, was used for comparison between multiple-exposure tests (the response of each fish was evaluated sequentially at four doses that increased after 20 minutes) and single-exposure tests (the response of each fish was evaluated after exposure to a single concentration).*

Steep-gradient multiple exposure				Steep-gradient single exposure			
Number of fish tested[a]	Concentration (μg/liter)	PC_{time}	$PC_{activity}$	Number of fish tested	Concentration (μ/liter)	PC_{time}	$PC_{activity}$
			Copper				
8	2.9 (1.1)	0.09 (0.20)	0.24 (0.21)	5	3.2 (1.1)	−0.03 (0.18)	0.53 (0.87)
	5.8 (1.1)	−0.63 (0.13)	−0.45 (0.23)	4	3.2 (1.2)	−0.31 (0.14)	0.13 (0.16)
	9.5 (1.1)	−0.85 (0.11)	−0.89 (0.07)	5	5.2 (1.1)	−0.65 (0.12)	−0.45 (0.19)
	17.2 (1.1)	−0.89 (0.05)	−0.89 (0.05)	5	10.7 (1.1)	−0.52 (0.11)	−0.10 (0.21)
			Nickel				
9	6.3 (1.1)	−0.06 (0.11)	−0.18 (0.09)	9	4.4 (1.1)	−0.21 (0.08)	−0.18 (0.14)
	15.2 (1.1)	−0.33 (0.14)	−0.44 (0.08)	8	20.7 (1.1)	−0.27 (0.24)	−0.21 (0.30)
	35.3 (1.1)	−0.76 (0.06)	−0.76 (0.06)	10	34.6 (1.0)	−0.65 (0.07)	−0.61 (0.08)
	103.5 (1.0)	−0.87 (0.06)	−0.86 (0.06)	9	85.9 (1.0)	−0.81 (0.04)	−0.68 (0.08)

[a] The same fish were tested at each exposure concentration of copper or nickel in multiple-exposure tests.

metal tests were compared at each time interval (20, 40, 60, and 80 minutes) by a two-sample *t*-test that does not assume equal variances (Steel and Torrie 1960). At 20 minutes there were no significant differences in the PC_{time} or $PC_{activity}$ between metal and control tests (Table 2). At 40 minutes, however, there was a significant decline in the PC_{time} for both copper and nickel exposures (copper = 5.8 μg/liter; nickel = 15.2 μg/liter). Significant declines in the $PC_{activity}$ as compared with control values occurred at 60 and 40 minutes for copper and nickel exposures, respectively (copper = 9.5 μg/liter and nickel = 15.2 μg/liter) (Table 2).

Shallow-Gradient Tests

Rainbow trout avoided copper and nickel at concentrations of about 4.4 and 23.4 μg/liter, respectively, when exposed under shallow concentration-gradient conditions (Fig. 4). In copper tests, fish appeared to detect concentrations down to 1.4–2.7 μg/liter, because declines in residence time started to occur at these lower concentrations (Table 3). Rainbow trout also responded to nickel at very low concentrations, but rather than avoidance, attraction to concentrations of about 6 μg/liter was observed. Avoidance only occurred when concentrations reached 10–19 μg/liter. The slight increase in residence time noted at about 60 μg/liter nickel (78 seconds), was only 55% of the control value for this area of the chamber, indicating avoidance was occurring even though the residence time did not drop below 50 seconds.

Activity in treated zones was closely correlated to the percent residence time in both copper

TABLE 2.—*Means (SE) of the proportional changes in residence time (PC_{time}) and activity ($PC_{activity}$) of rainbow trout during control tests (no toxicant added) and copper and nickel multiple exposures in steep-gradient tests compared over discrete time intervals following toxicant addition to the avoidance chamber. The PC_{time} and $PC_{activity}$ values for the copper and nickel tests correspond to those values reported in Table 1. Statistical comparisons between metal and control tests for each time interval were evaluated by a two-sample t-test that did not assume equal variances (Steel and Torrie 1960). Asterisks denote statistically significant differences from control values: P ≤ 0.05*, 0.01**, or 0.001***.*

	Control tests			Copper tests			Nickel tests		
Time (minutes)	Number of fish tested	PC_{time}	$PC_{activity}$	Number of fish tested	PC_{time}	$PC_{activity}$	Number of fish tested	PC_{time}	$PC_{activity}$
20	20	−0.05 (0.10)	−0.16 (0.08)	8	0.09 (0.20)	0.24 (0.21)	9	−0.06 (0.11)	−0.18 (0.09)
40		0.16 (0.09)	−0.16 (0.09)		−0.63 (0.13)***	−0.45 (0.23)		−0.33 (0.14)**	−0.44 (0.08)*
60		0.07 (0.08)	−0.24 (0.08)		−0.85 (0.11)***	−0.89 (0.07)***		−0.76 (0.06)***	−0.76 (0.06)***
80		−0.14 (0.08)	−0.31 (0.06)		−0.89 (0.05)***	−0.89 (0.05)***		−0.87 (0.06)***	−0.86 (0.06)***

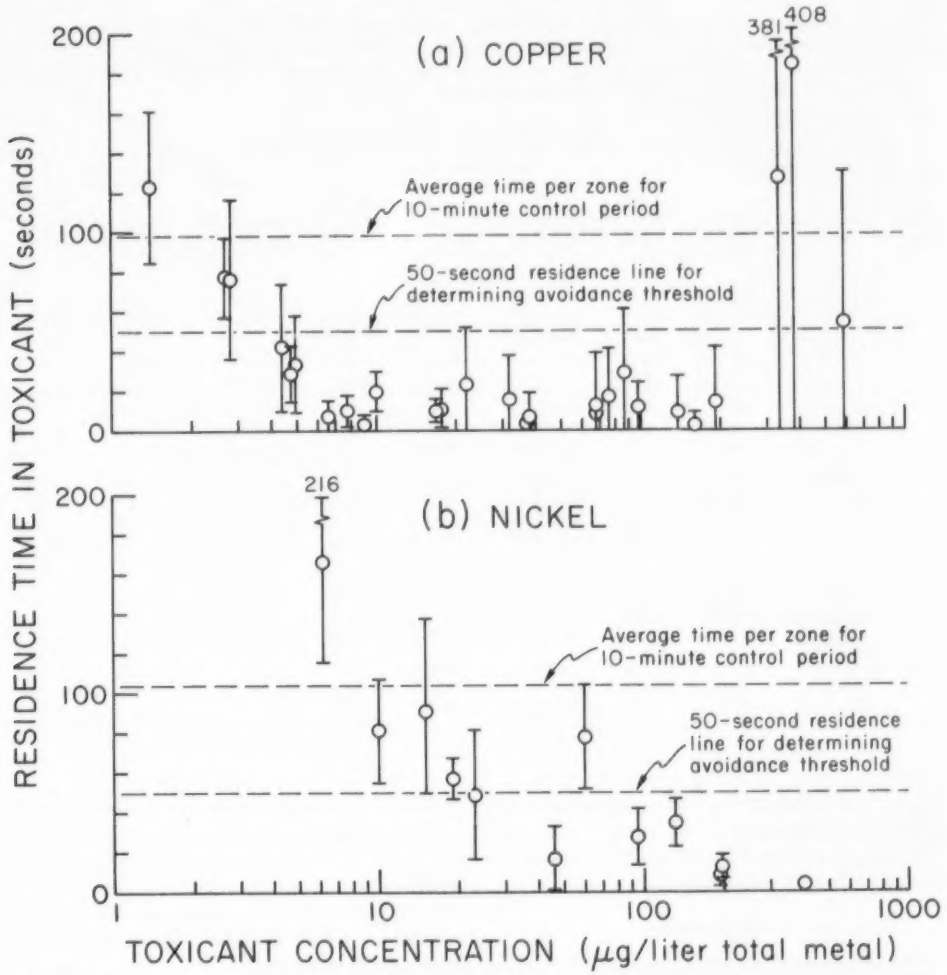

FIGURE 4.—*Residence time (mean ± 2 SE) of rainbow trout corresponding to concentrations of copper (a) and nickel (b) in shallow-gradient tests. The average time per zone for the control period is the average of control residence times given in Table 3 for each toxicant. Residence times below the 50-second residence line were taken to indicate significant (non-statistical) avoidance of the toxicant concentration. The number of fish tested and numerical results are given in Table 3.*

and nickel tests. At approximately 6 μg/liter nickel, however, when a 40% increase in residence time over control values was noted, only a slight increase in activity occurred (Table 3). A general decrease in the swimming speed of the fish or an increase in the frequency of turns in this zone would account for this observation. Activity in the other treated zones, however, remained relatively high (26 and 19 blocks/gate at 10 and 19 μg/liter, respectively; Table 3).

Due to experimental error in mixing the copper stock solution, four fish were exposed to exceedingly high copper concentrations. Results indicated initial avoidance at lower concentration, but diminished avoidance and, in some instances, apparent attraction to higher copper concentrations (Table 3; Fig. 4). Although apparent attraction occurred at concentrations of 334 and 386 μg/liter, activity within these regions increased only slightly and was still well below control values (Table 3). Activity in adjacent concentrations also remained relative-

TABLE 3.—*Summary of mean (SE) toxicant concentrations, residence time, and activity counts for each area of the avoidance chamber during shallow concentration gradient exposures. The low-concentration area corresponds to the zone adjacent to the center drain, whereas the high-concentration area is the zone on the end of the tank where toxicant first enters (Fig. 1). ILED = infrared light-emitting diode. Asterisk (*) denotes control.*

Number of fish tested	Area on exposure side of avoidance chamber								
	Low concentration			Medium concentration			High concentration		
	Concentration (μg/liter)	Time (seconds)	Activity (blocks/ ILED)	Concentration (μg/liter)	Time (seconds)	Activity (blocks/ ILED)	Concentration (μg/liter)	Time (seconds)	Activity (blocks/ ILED)
					Copper				
7	<2.0*	138 (17)	34 (4)	<2.0*	121 (16)	28 (3)	<2.0*	55 (10)	18 (3)
	1.4 (0.2)	123 (19)	33 (5)	2.7 (0.5)	77 (10)	18 (2)	4.8 (0.9)	34 (12)	15 (5)
	2.8 (0.3)	76 (20)	23 (7)	4.8 (0.8)	29 (7)	8 (2)	6.6 (0.6)	7 (4)	3 (1)
	4.4 (0.6)	42 (16)	9 (4)	7.8 (1.4)	10 (4)	3 (2)	9.0 (0.7)	4 (2)	1 (0.5)
7	<2.0*	100 (10)	53 (8)	<2.0*	99 (12)	43 (8)	<2.0*	87 (33)	43 (15)
	10.1 (1.4)	20 (5)	12 (3)	17.0 (1.4)	10 (3)	7 (2)	36.8 (1.8)	4 (2)	5 (2)
	17.6 (1.9)	11 (5)	6 (4)	37.8 (3.2)	7 (6)	4 (3)	76.3 (4.4)	17 (12)	4 (3)
	32.1 (3.8)	16 (11)	5 (3)	68.1 (7.2)	13 (13)	4 (4)	139.1 (8.3)	9 (9)	5 (5)
4	<2.0*	99 (10)	54 (6)	<2.0*	111 (12)	51 (6)	<2.0*	65 (16)	32 (6)
	22.3 (7.1)	24 (14)	18 (9)	99.1 (7.4)	12 (6)	8 (4)	160.3 (6.3)	3 (3)	2 (2)
	67.9 (14.1)	8 (6)	5 (4)	191.2 (2.1)	14 (14)	8 (8)	333.6 (20.6)	127 (127)	10 (9)
	87.2 (31.0)	29 (16)	10 (7)	386.1 (74.6)	184 (112)	18 (8)	589.9 (15.4)	54 (38)	15 (11)
					Nickel				
5	<0.1*	118 (20)	39 (5)	<0.1*	82 (11)	32 (6)	<0.1*	94 (19)	26 (4)
	6.2 (0.9)	166 (25)	42 (8)	10.1 (1.4)	81 (13)	26 (6)	19.0 (2.9)	57 (5)	19 (5)
	15.2 (1.2)	91 (23)	24 (7)	23.4 (3.6)	49 (16)	12 (4)	45.8 (4.9)	17 (8)	6 (3)
10	<0.1*	141 (14)	45 (8)	<0.1*	102 (20)	29 (6)	<0.1*	86 (13)	28 (5)
	60.2 (3.5)	78 (13)	24 (6)	94.6 (4.8)	28 (7)	11 (5)	196.1 (5.4)	9 (3)	5 (2)
	131.5 (6.0)	35 (6)	12 (3)	199.0 (7.8)	13 (3)	4 (2)	402.7 (9.1)	4 (2)	3 (2)

ly low, indicating slower swimming speeds and declining movements in the treated zone. Attraction at relatively high concentrations was not observed during nickel tests.

Discussion

No differences in the avoidance thresholds to copper or nickel were found between the various methods of exposure we analyzed. The avoidance thresholds determined for copper (4.4 μg/liter) and nickel (23.4 μg/liter) with the shallow gradient fell well within the 95% CL of the avoidance threshold for the multiple-exposure steep gradient (2.6–15.5 and 10.2–54.3 μg/liter, respectively). In the shallow gradient, fish were confronted by ascending toxicant concentrations in a limited area (one-half the length of the avoidance chamber). This may better represent actual field conditions than steep gradients in some cases, but the latter yielded virtually identical results. This factor, combined with the relative ease with which the steep-gradient avoidance response can be quantified and statistically analyzed, leads us to believe that the steep gradient would be the simplest exposure method for determining avoidance responses. Furthermore, exposure of individual fish to several increasing concentrations is a valid test procedure when short time intervals (20 minutes or less) are used.

It generally has been accepted that avoidance of a toxic solution depends not only on the magnitude of the concentration, but also on the rate of change in toxic concentrations between two areas of water (Ishio 1965). The locomotor response of the goldfish *Carassius auratus* depended on the slope of the gradient of the copper ion when all other conditions were similar (Kleerekoper et al. 1972; Timms et al. 1972; Westlake et al. 1974). Also, Westlake et al. (1974) reported that avoidance may result when exposure to a copper concentration occurs through a steep gradient, but attraction to a similar concentration can result if exposure occurs through a shallow gradient. Our tests were not designed to specifically test for gradient-slope effects on

the avoidance response; the slope of the gradient between control and treated water increased as the toxicant concentrations rose, making it impossible to distinguish slope effects from absolute concentration effects. The primary question addressed in our study was, would a fish swimming upstream into a toxic effluent eventually avoid a concentration similar to that causing avoidance had the fish swam directly into it, bypassing lower concentrations? Our results indicate that rainbow trout, under these test conditions, would avoid similar concentrations of copper and nickel. Our results also agree with those of Kleerekoper et al. (1972), in that under some conditions at very low copper and nickel concentrations, rainbow trout were attracted to the toxicant.

The strong attraction response to high copper concentrations following initial avoidance of lower concentrations cannot be clearly explained, but is not incongruous with results presented by other authors. Black and Birge (1980) reported that rainbow trout avoided 0.07 mg/liter (the lowest concentration tested), but were attracted to copper at 4.6 and 7.6 mg/liter. Maciorowski et al. (1977) found that the aquatic invertebrate *Gammarus lacustris* avoided concentrations of 0.15 and 0.46 mg/liter Cu^{++}, but attraction occurred at 12.3 and 30.0 mg/liter. Lake whitefish *Coregonus clupeaformis* avoided 0.05 mg/liter Cu^{++}, but were attracted to 1, 5, and 30 mg/liter (Hara and Scherer unpublished data as reported by Maciorowski 1977). Causes of attraction previously have been related to narcotic effects (Jones 1947, 1948) or to changes in preference-avoidance behavior at different concentrations resulting from changes in the sensitivity of the chemoreceptors (Sprague and Drury 1969; Hara et al. 1976). No indication of possible narcosis induced by nickel was evident in this study and, although the reduced activity coupled with strong attraction found at the high copper concentrations apparently indicated immobility, this was not conclusive.

Sprague (1964) reported avoidance thresholds for Atlantic salmon parr *Salmo salar* of approximately 2 μg/liter copper in water that had a total hardness of 18 mg/liter. Field concentrations of copper (17–21 μg/liter) and zinc (210–258 μg/liter) have been found to cause downstream movements of Atlantic salmon, and equitoxic copper and zinc mixtures above 38 and 480 μg/liter, respectively, appeared to prevent upstream migrations of this species (Sprague et al. 1965). Westlake et al. (1974) reported goldfish avoidance of copper in a steep-gradient, circular tank at concentrations equal to or greater than 5 μg/liter (water hardness = 5.4 mg/liter). In earlier tests conducted at WFTS, also in a circular tank, rainbow trout avoided copper concentrations of about 4 μg/liter (Donald G. Stevens, personal communication, Western Fish Toxicology Station). These results indicate that the copper avoidance thresholds we report based on the computer-based data acquisition system are in close agreement with previously reported values. Furthermore, there appears to be little difference between results generated in linear or circular avoidance chambers. Although considerable data were available on nickel toxicity, no previous studies were found that quantified avoidance responses to this metal.

Ambient water-quality criteria have been published by the USEPA for both copper and nickel. For total recoverable copper, the criterion for protecting freshwater aquatic life is 5.6 μg/liter as a 24-hour average (USEPA 1980b). The avoidance threshold we determined was 6.4 μg/liter copper, indicating that rainbow trout would avoid this metal at levels previously believed adequate for protecting freshwater biota, and below most chronic toxicity values reported for copper (USEPA 1980b). McKim and Benoit (1971) reported that the maximun. acceptable toxicant concentrations (MATC) for brook trout *Salvelinus fontinalis* exposed to copper fell between 17.4 and 9.5 μg/liter (water hardness = 45 mg/liter as $CaCO_3$). Changes in cough frequency, locomotor activity, and feeding behavior, for the same species, were found to occur at copper concentrations of 6–15 μg/liter (Drummond et al. 1973), which correlates well with our avoidance concentrations for rainbow trout. Hara et al. (1976) reported that the threshold concentration of copper required to cause a minimal depression in the bulbar response of rainbow trout to the olfactory stimulant L-serine was 8 μg/liter. Depression of the bulbar response increased at higher concentrations, eventually causing apparent irreversible damage to the olfactory receptors at 50 μg/liter. Hara (1972) postulated that this blocking effect was caused by cupric ion interference with either the receptor sites or enzymatic components of the receptor membranes. On two estuarine

species, Gardner and LaRoche (1973) found physical damage of the olfactory chemoreceptor sites at the lowest copper concentrations they tested (50 μg/liter). They concluded that brief encounters of fish with toxicants such as copper, while not causing immediate death, perhaps "... could indirectly promote lethality by disrupting a species' ability to perceive peripheral events." Our results indicate that avoidance to copper most likely occurs at concentrations below those found to depress olfactory responses, and certainly below those shown to cause physical damage. This behavior, therefore, would be of obvious adaptive value to a mobile organism, such as rainbow trout, encountering elevated copper concentrations under field conditions. The limited data available indicate that avoidance of copper under natural stream conditions does occur, and that this behavior can be an important factor influencing the migration, distribution, and survival of certain fish species (Sprague et al. 1965; Sutterlin and Gray 1973; Geckler et al. 1976).

For total recoverable nickel, the criterion for protecting freshwater aquatic life is based on a sliding scale determined by the hardness of the water being considered. The 24-hour average concentration should not exceed the numerical value given by $\exp[0.76 \log_e(\text{hardness}) + 1.06]$ (USEPA 1980c). In our study, the minimum hardness measured was 21 mg/liter as $CaCO_3$, which is typical for the Willamette River system during summer months (Samuelson 1976). The 24-hour average nickel concentration for these waters, therefore, should not exceed 29 μg/liter. Once again, our nickel avoidance threshold of 23.9 μg/liter closely approximated the safe level and was well below chronic toxicity values reported by the USEPA (1980c).

Behavioral toxicology testing has been viewed from a variety of standpoints. At the Corvallis Environmental Research Laboratory, we are examining the feasibility of using the avoidance test to screen new or existing chemicals. The results of such a test would be a valuable addition to the data used in setting water-quality criteria because, as our study indicates, important information not readily available from standard toxicity testing procedures can be gained from laboratory avoidance testing. The computer-based system described here offers a rapid, easy-to-operate, accurate, and relatively inexpensive means of collecting such data.

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